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# *A Genomic Approach to Investigate Resistance Mechanisms in the Two-Spotted Spider Mite* Tetranychus Urticae

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# Summary

The two-spotted spider mite (*Tetranychus urticae*) is a herbivore with an incredibly large host plant range. They are an economically important pest of field and greenhouse crops. Control of spider mites is mainly achieved by acaricide application. However, due to their favorable life-table parameters, spider mites rapidly develop resistance to these acaricides. Knowledge on the mode of action of acaricides and resistance mechanisms in spider mites is limited, especially when compared to insects, due to the absence of a chelicerate model system. Recently, the complete genome of *T. urticae* was sequenced and this opened the door for new and innovative techniques to study resistance in spider mites, some of which are implemented in this thesis.

In the first part, I investigated the unknown mode of action of the acaricides etoxazole, hexythiazox and clofentezine which all inhibit mite growth. In **Chapter II**, We used an etoxazole-resistant field strain (EtoxR), showing high levels of resistance, typically a feature of target site resistance. Crossing etoxazole susceptible and resistant mites revealed that resistance is recessive and under the control of a single gene. We developed a bulk segregant analysis (BSA) mapping method in combination with high-throughput sequencing to identify the genomic region involved with etoxazole resistance. Resistance could be located to a small genomic region corresponding with the chitin synthase-1 (*CHS-1*) gene. By analyzing *CHS-1* sequences of geographically distinct etoxazole-resistant strains, we found that a single non-synonymous mutation (I1017F) is involved in resistance to etoxazole. In **Chapter III**, this research was taken further to include hexythiazox and clofentezine. Based on a toxicology and cross-resistance pattern similar to etoxazole, it was suggested that the three acaricides share the same target site. We isolated a field-collected strain with recessive, monogenic resistance to all three compounds. We slightly adapted our BSA analysis and used one resistant male to found our segregating population. We found that the same genomic region (*CHS-1*) and non-synonymous mutation (I1017F) are involved with hexythiazox and clofentezine resistance. The I1017F mutation is located in the last transmembrane helix of CHS-1, which is proposed to be involved with pore formation and chitin translocation. Based on our experiments, we suggest that etoxazole, hexythiazox and clofentezine bind to that region and block chitin translocation.

In second part of this thesis, we investigated the molecular mechanisms of spirodiclofen resistance in two genetically distant strains. In **Chapter IV**, we first investigated the spirodiclofen-resistant strains for cross-resistance to spiromesifen and spirotetramat, members of the same acaricidal group as spirodiclofen. Whereas the levels of cross-resistance were low, they indicated a common detoxification pathway. We sequenced the complete resistant acetyl-

coA-carboxylase (*ACCase*) gene and found no fixed non-synonymous mutations. Furthermore, there was no alteration in expression levels, ruling out target site resistance. A whole genome-wide expression microarray based on the *T.urticae* genome sequence was developed to compare gene expression levels between the spirodiclofen-susceptible (LS-VL) and -resistant spider mite strains (SR-VP and SR-TK). Despite the different genetic background of the spirodiclofen-resistant strains, we observed a similar response of differentially expressed genes. Most of the genes identified belonged to the classical detoxification families (P450s, CCEs and GSTs). **Chapter IV** subsequently focused on analyzing the overexpressed P450s. Through quantitative PCR, two P450s were identified (*CYP392E7* and *E10*) which were possibly involved in spirodiclofen metabolism. Functional expression of both genes revealed that only CYP392E10 metabolized spirodiclofen, but not its corresponding enol. The major reaction mechanism of CYP392E10 is the hydroxylation of the spirocyclic ring on the spirodiclofen substrate. CYP392E10 was also found to be responsible for hydroxylation of spiromesifen. However, metabolism of spirotetramat could not be shown.

For **Chapter V**, we revisited the results of the microarray study and included an overexpressed CCE (*CCE04*) in our analysis. Validating the results of the microarray with quantitative PCR revealed a discrepancy from the sequenced CCE04 gene of the genome. Sequencing of the *CCE04* gene of spirodiclofen-resistant strain (SR-VP) uncovered the presence of 2 *CCE04* alleles: one matching with the *CCE04* sequence of the London genome (named London-specific allele) and one with several point mutations (named SR-VP specific allele). The SR-VP specific allele codes for a protein with 31 amino acid substitutions compared to the London-specific allele. To determine gene copy number, we performed qPCR on gDNA of spirodiclofen-resistant (SR-VP and SR-TK) and susceptible (LS-VL and London) strains using allele-specific and unspecific (amplifying both alleles) primers. Quantitative PCR with allele-specific primers revealed an enrichment of the SR-VP-specific allele in the spirodiclofen-resistant strains. However, qPCR with the unspecific primers did not reveal a change in copy number and RFLP analysis of haploid males excluded gene duplication as a possible mechanism. Preliminary functional expression experiments revealed kinetic differences between both enzymes, and inhibition experiments revealed that the SR-VP-specific enzyme is less inhibited by spirodiclofen. We suggest that the resistant CCE04 enzyme delays hydrolysis of spirodiclofen to its active enol, allowing CYP392E10 to metabolize spirodiclofen.